MASSOSPORA CICADINA PECK

A Fungous Parasite of the Periodical Cicada

A. T. Speare

(With Plates 5 and 6)

Among the enemies of the periodical cicada, *Tibicina septendecim* (L.), none perhaps is of more interest than the fungus *Massospora cicadina*. It is of interest because to perpetuate itself upon a host of such extraordinary life habits, and so far as is known it occurs on no other host, it must likewise possess a very unusual mode of life. It is of interest also because its relationship to other entomogenous fungi has not been clearly understood up to the present time, and, like many other entomogenous forms, it is worthy of especial consideration because it attacks an insect of some economic importance.

Although the organism was apparently first observed by Leidy (1850), the first description of it was published by Peck (1879). It seems probable that Peck observed the resting spores of the fungus as well as its conidia, but apparently he did not observe the processes associated with the formation of either of these types of reproductive bodies, and, lacking the information that a study of such stages would have afforded him, the organism was erroneously placed near *Protomyces* among the *Coniomycetes*. Thaxter (1888) almost simultaneously with Forbes (1888), published a brief note in which the fungus was considered as a member of the Entomophthorales, but as only a few old dried specimens were available for study at the time, none of which showed the resting spores, he apparently did not feel fully justified in assigning it to this family of fungi.

In addition to the above mentioned papers, several others have appeared such as those of Butler (1886) and Marlatt (1907), in which the gross appearance of the fungus and of the diseased cicadas was briefly described, but with the exception of the above

mentioned brief note of Thaxter no other publication has appeared, as far as the writer is aware, in which the microscopic characters of the fungus have been considered.

Like the host which it parasitizes, *Massospora cicadina* is, so far as is known, peculiar to America, and as a result, Europeans, to whom the fungus is known only by such fragmentary and incomplete references as those noted above, have been more or less confused in regard to the nature of the organism, Lakon (1919a), for example, classing it with *Sorosporella agrotidis* Sor. (*Sorosporella uvella* (Krass.) Gd.) and *Massospora staritzii* Bresadola as "Unvollkommen bekannte Entomophthoreen bezw. als solche beschriebene Pilze."

During the summer of 1919, Brood X of the periodical cicada made its appearance in the vicinity of Washington, D. C., and an excellent opportunity was thus afforded the writer to study its fungous parasite. The later was first observed on May 31, about ten days after the first insects emerged from the earth, and from this date until the disappearance of the brood in the early part of July it was constantly present, though in no great abundance until after June 10.

The resting spore as well as the conidial condition of the fungus was common about Washington, in 1919, but the latter was never as abundant as the former, and while it was often a difficult matter to collect during an afternoon a dozen cicadas showing conidia, during as many hours later in the season it was not difficult to collect hundreds of specimens showing the resting spores. It should be noted, that both types of reproductive bodies were never found either simultaneously or consecutively in the same individual, and it was determined that the conidia and the resting spores occurred at different periods in the aërial life of the host, the former appearing exclusively in the early part of the season, the latter developing toward the end of the aërial existence of the insect. It should be noted furthermore that the fungus seemed to be largely though not exclusively confined to the male insects. Despite the fact that infected insects were observed and collected many times during the season, not more than half a dozen parasitized females were observed. Whether or not the

disproportionately large numbers of infected male individuals indicates a predisposition of the latter to attack by *Massospora cicadina* has not been determined but the present instance is not the only one of the kind for Giard (1888) records the same phenomenon in connection with a fungus upon *Tipula paludosa*, which he appropriately called *Entomophthora arrenoctona*.[1] Nevertheless it is a rather unusual condition and one that has not yet been satisfactorily explained. Not only is present fungus largely confined to male insects but in the resting spore condition at least, it seems furthermore, to parasitize spent individuals in most instances. In the closing days of the brood, when the females were busy ovipositing in the tree tops, it was observed that simultaneously, the males occurred by hundreds, either dead upon the ground, or alive and feebly attempting to crawl from the ground up the trunks of trees. A very large percentage of such males were found upon examination to show the fungus parasite in some stage of resting spore development. It seems reasonable to conclude, as the large numbers of dead and dying males were found at a time when the females were laying eggs, that fertilization of the females had taken place in most instances and that the dead and dying males were largely spent individuals. It is not possible, however, to state whether or not the dead male insects found in early, or mid-season, in which it will be recalled conidia only occurred, had mated, but in any event such individuals were relatively few in numbers.

An examination of the healthy as well as the infected male insects, particularly toward the end of the brood, showed that the anterior portion of the abdomen was invariably empty. The genitalia and nearly all of the other internal organs were concentrated in the last four or five segments of the abdomen. This condition was also observed by Mr. R. E. Snodgrass of the Bureau of Entomology, who found furthermore that a sac was

[1] It is perhaps appropriate at this time to point out that Dr. Roland Thaxter, of Harvard University, who possesses the type of *Entomophthora arrenoctona* Giard, believes this fungus to be identical with *Entomophthora caroliniana* (Thaxt.). Although both descriptions were published in 1888, that of Thaxter appeared in April, and that of Giard some time after July 11. Hence the name *Entomophthora caroliniana* (haxt.) is the correct one and should be used for the fungus in question.

formed in the anterior portion of the abdomen which upon enlargement and inflation pushed the genitalia to the position indicated, and also pushed the intestine which normally in most insects lies close to the ventral abdominal wall to a position upon the dorsal wall. This sac becomes so large that it occupies the greater part of the abdomen, and in the opinion of Mr. Snodgrass it may act as an air reservoir in both sexes, and in addition, in the male, have a resounding function for the stridulatory apparatus. In any event a portion of the wall of this sac forms a septum across the body cavity, effectually separating the genitalia and other organs from the empty anterior portion of the abdomen, and the fungus which lives entirely upon the softer tissues of the insect's body is therefore limited in its development to the last four or five segments of the body in which the genitalia and other similar organs are concentrated.

As the conidial and the resting spore conditions do not occur simultaneously in the same individual and as the insects in which conidia are formed present quite a different appearance from those in which resting spores occur, it seems advisable to consider each phase of development separately.

CONIDIAL DEVELOPMENT

Infected individuals showing the conidial stages of the fungus appear in a way such as is illustrated on Plate 5, Fig. 1.[2] Specimens such as those shown, were usually found lying dead upon the ground beneath trees, or in open roadways, although very often a similarly afflicted cicada was observed flying around in an unsteady manner, or crawling feebly about. Unfortunately no specimens showing an earlier phase of the disease were collected, and therefore while the method of formation of the conidia was followed in several instances, an earlier stage homologous to the "hyphal body" stage of other Entomophthorales was not observed.

The fungus thus confined in its vegetative growth to the softer

[2] In the specimens shown the wings and legs were removed artificially in certain instances, in order better to expose the fungus mass for photographic purposes, and in the individuals shown on Plate 5, Fig. 2, a portion of certain of the abdominal rings was removed for the same purpose.

tissues in the posterior segments of the body of the host, ultimately destroys all such tissues, including the flexible intersegmental membranes of the abdomen in this region. As a result of the complete destruction of these membranes the posterior abdominal segments slough off until a condition such as that illustrated on Plate 5, Figs. 1 *B* and *C* is reached. The sloughing off process takes place progressively, beginning with the last segment and continues until four or more have been dropped, the last remaining one marking the position of the septum referred to above. The insect does not die at the time the first segments are dropped. On the contrary it remains alive for a considerable period and continues to fly and crawl about from place to place.

As far as the writer is aware such a sloughing off process, taking place while the host is alive, is quite unknown in other insects attacked by other members of the Entomophthorales, and in fact the phenomenon is so unusual that it has been noted by practically every person who has observed the disease in the field. The appearance of insects crawling and flying about with but two or three abdominal segments attached to the thorax, is indeed sufficiently striking to attract the attention of any one.

The fungus mass, including the conidia, which morphologically is of endogenous origin, becomes exposed as fast as the body segments of the host rot away, and the movements of the insect from place to place serve to disseminate the conidia in a way that could scarcely be improved by any natural method. It will be recalled that in most of the entomogenous entomophthorales, the conidia are borne upon conidiophores which bore their way outward through the body wall of the host, and that they are violently ejected from the conidiophores only after the host is dead and therefore stationary. Although the conidia are thrown to some distance, such a method seems inefficient when it is compared with the process which takes place in the present instance, in which the live, actively moving infected host mingles promiscuously with its fellows.

The fungus when intact forms a clay colored pustule like, granular mass at the tip of the abdomen. In certain individuals such as is shown on Plate 5, Fig. 1 *C* the pustule is quite large,

assuming the size and conformation of that part of the abdomen which it formally occupied. In other specimens Plate 5, Fig. 1 *B* and *D,* the pustule is asymmetrical and ragged. Such specimens as the latter are evidently old ones, from which a large part of the conidia were detached when the hosts were alive and moving about. Upon microscopic examination the pustule is found to be composed almost wholly of conidia, although if search is made deep within the mass close to the septum, conidiophores and the characteristic entomophthoroid hyphal fragments may also be seen.

As noted above, in the species of Entomophthora, the conidia are violently discharged from the conidiophores. In *Massospora,* however, the conidia are formed within the body of the host, and although they are cut off in the usual manner their ejection is prevented by the body wall of the insect, which when they are cut off is intact, and holds them in the approximate position in which they are produced. The conidia therefore cohere with one another and a mass is formed which upon disintegration of the intersegmental abdominal membranes is exposed, and assumes the form of a pustule such as is described above. The movement of the host at this period is perhaps the most important factor in loosening the segments of the abdomen, the membranes connecting which have been destroyed by the vegetative development of the fungus so that the movements of the insects not only serve to scatter the conidia of the fungus, but first free them from captivity.

The conidia are, so far as the writer has been able to determine, all of one type, which conforms in most respects to that of the other Entomophthorales. They are quite regularly oval in form, measuring 10–14 × 14–17 microns. The papilla, an outgrowth characteristic of the conidia of all members of the family, is usually not prominent, though always noticeable. Occasionally it stands out conspicuously in a manner such as is shown on Plate 6, Fig. *A*. Unlike other members of the family, however, the conidial walls are regularly verrucose, which condition renders them unique in appearance. It should be noted, however, that there is a tendency for them to lose the warted appearance if they are permitted to remain in water for a short time.

The method of formation of the conidia and the manner in which they are cut off seems quite like the analogous processes in other species and need not be discussed here.

When viable conidia were placed upon a slide in a moist chamber, or when they were sewn upon a nutrient agar, germination usually took place in a manner such as is illustrated on Plate 6, Figs. 2–3, namely, by one or more rather stout, long, germ tubes. Occasionally, however, a single rather stout germ tube arose, the terminal portion of which became swollen, Plate 6, Fig *D,* as though to form a secondary conidium, but at this point development invariably ceased.

In connection with the germination tests, attempts were made to grow the fungus artificially. The media used were potato agar, Molische's agar, oat agar, and nutrient beef broth. In addition to these nutrients, the genitalia and other organs, upon which the fungus normally grows in nature, were removed aseptically from live, healthy cicadas and employed without sterilization, for the same purpose. No growth of the fungus was obtained, however, upon either the unsterilized tissues from freshly killed insects, or upon the other nutrients noted above.

The conidia when placed in a suitable situation germinate with great rapidity, a growth such as that illustrated on Plate 6, Fig. *B–C,* taking place within three hours, but after such a short, rapid, preliminary growth development ceased in every instance in the writer's tests.

RESTING SPORE DEVELOPMENT

Up to the present time resting spores have not been definitely associated with the organism in question, although Peck (1879) vaguely described bodies, which Thaxter (1888) subsequently tentatively regarded as resting spores. In the light of these investigations furthermore, it likewise appears that many of the early notes about the fungus contain references to the resting spore condition, although the descriptions were of such a nature that they might have applied equally well to the conidial growth.

As noted above the resting spore condition, which was never found associated with the conidial condition, was very prevalent

about Washington in 1919, from 50–90 per cent of the male insects showing this stage of the fungus during the latter part of the season.

In its vegetative growth prior to the production of resting spores, the fungus destroys the intersegmental abdominal membranes of the host, as it does in the conidial phase of the development just considered, and there is a similar sloughing off of the abdominal segments. The septum described above, across the body cavity of the insect, which normally persists in insects affected with the conidial growth is, however, destroyed in most instances during the formation of the resting spores, and although these bodies arise upon the soft tissues concentrated in the last four or five posterior segments of the body, they may be found, owing to the absence of the septum, in some numbers, within the otherwise empty anterior portion of the abdomen.

The resting spore-mass which is, nevertheless, largely confined to the posterior segments, presents a granular appearance and is of a sulphur yellow color, tinged with green when young, but it assumes a dark brown color when the resting spores are mature. These bodies are less coherent in the mass than are the conidia, and as a result they are scattered about by the movements of the host much more freely. It was in fact not uncommon to observe an infected individual in which the empty body cavity formed one continuous passage from the last abdominal segment to the head, with two or three of its abdominal segments missing, actively crawling or flying about. In this respect the appearance of cicadas showing the resting spores, differs from those showing the conidial growth, because it will be recalled there occurs in the latter a persistent fungus stroma closely associated with the above-mentioned septum, which after the abdominal segments have been dropped, remains as a continuous partition across the abdomen.

It can therefore be readily seen that, though both of the reproductive phases have many characteristics in common, there are nevertheless certain characters by which one phase may be readily distinguished from the other merely by a superficial examination.

Microscopically the mature resting spores, or as they perhaps should be called, azygospores, appear as spherical, slightly brownish bodies, the outer wall of which is beautifully reticulated in a manner such as is shown on Plate 6, Fig. *T*. They are remarkably uniform in size, mesauring 38–48 microns in diameter, averaging 44 microns.

Unfortunately all stages in the development of these azygospores were not seen in fresh material and particularly those stages associated with the transfer of protoplasmic material from the byphal body to the resting spore. Alcoholic material, which it may be stated was all collected in the daytime, indicates, however, that the process is a non-sexual one, and that the azygospores arise as buds or outgrowths upon the hyphal bodies into which, as they enlarge, flows the entire protoplasmic contents of the hyphal body, the empty and evanescent walls of which sometimes remain attached to the mature resting spores.

The writer showed (Speare, 1912) in connection with *Entomophthora pseudococci* that the presence or absence of daylight, at the time of maturity of the hyphal bodies, predetermined to a large extent the type of reproductive body that was formed, and that the azygospores of the fungus in question, could be produced at will, by placing artificial cultures of the fungus in a dark situation a few hours before the hyphal bodies were ready to "germinate." It would therefore seem reasonable, if one desired to collect the early resting spore stages in such a similar form as *Massospora cicadina,* to search for them during the night, yet, inadvertently no collections were made at this time in the present investigation. Nevertheless, the alcoholic material shows with reasonable certainty that no sexual process is present, and that the development of the resting spores, conforms quite well with the development of the azygospores in other members of the family such as *Entomophthora aulicae* Reich.

The resting spores of *Massospora cicadina* like the analogous bodies of many other of the entomogenous species of the family have never been seen to germinate. In the writer's tests a number of them were heated at varying degrees of temperature, and a number were permitted to remain out-of-doors all winter, yet no

germination was observed, when, after such treatment they were suspended in a drop of water in Van Tieghem cells. Similar negative results were obtained in attempts to germinate resting spores that had previously been treated with dilute hydrochloric acid for a short time.

The writer has obtained no information in these studies regarding the manner by which *Massospora cicadina* passes the 16 years and 9 months' subterranean existence of its host. That it lives during this period either on the larvae of *T. septendecim,* or on other similar biennial cicadas seems the reasonable supposition, yet there is no evidence at hand to support this theory. It is probable that when it has been determined how, for example, *Entomophthora muscae* and other species that are not known to form resting spores, live over winter (see Lakon, 1919 *b*), information will be at hand that will be of value in solving the peculiar conditions involved in the present instance.

From the economic viewpoint it must be stated that if the fungus is confined largely to spent males and does not attack and kill the larvae (the writer observed it only on adult individuals), its importance as a natural check to the spread of this insect is almost negligible. Investigation should be made, however, of larvae two or three years before their emergence in order to determine whether or not the fungus is present.

These studies show, it is hoped, that there can no longer be any question regarding the relationship of *Massospora cicadina* to other entomogenous Entomophthorales, and that while it is a very distinct form in many respects, it falls quite naturally into the above mentioned family.

BUREAU OF ENTOMOLOGY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

LITERATURE CITED

Butler, A. W. 1886. The periodical cicada in Southwestern Indiana. Bul. 12, Div. Ent. U. S. Dept. Agri., July, 1886, p. 24.

Forbes, S. A. 1888. On the present state of our knowledge concerning insect diseases. Psyche, Vol. V, p. 3.

Giard, A. 1888. Fragmentes Biologiques XI, Sur Quelques Entomophthorees. Bull. Scient. de la Fr. et Belg. Tome, XIX, p. 298.

Lakon, G. 1919*a*. Die Insektenfeinde aus der Familie der Entomophthoreen. Zeit. f. Angew. Entom., Bd. V, 1919, p. 186.

Lakon, G. 1919*b*. Bemerkungen über die Überwinterung von *Empusa muscae*. Zeit. f. Angew. Ent., Bd. V, p. 287, 1919.

Leidy, J. 1850. (Note on fungus disease of *Cicada septendecim*.) Proc. Acad. Nat. Sci. Philadelphia, Vol. 5, 1850–51, p. 235.

Marlatt, C. L. 1907. The periodical cicada. Bull. 71, Bur. of Ent. U. S. Dept. Agri.

Peck, C. 1879. *Massospora cicadina* n.g. et sp. Thirty-first Report of State Botanist of New York, p. 44.

Speare, A. T. 1912. Fungus parasites of insects injurious to sugar cane. Bull. 12, Path. Ser. Hawaiian Sugar Planters' Experiment Station, Honolulu, Hawaii.

Speare, A. T. 1919. The fungus parasite of the periodical cicada. Science, n.s., Vol. I, No. 1283, p. 116, August, 1919.

Thaxter, R. 1888. The Entomophthoreae of the United States. Mem. Bost. Soc. Nat. Hist., Vol. IV, No. VI, p. 190.

EXPLANATION OF PLATES

PLATE 5

Fig. 1. Specimens of *Tibicina septendecim* showing the conidia of *Massospora cicadina*. Although certain organs of these insects were removed artificially, the abdomen with the attached fungus mass is shown in each instance exactly as it was observed in the field. Fig. 1*A* is a female individual and shows an unusually large conidial mass. × 1.

Fig. 2. Specimens of *Tibicina septendecim* showing the resting spores of *M. cicadina*. In Fig. 2*A* a portion of the anterior four abdominal segments were removed artificially. Fig. 2*B* shows the fungus mass within the abdomen, viewed from a posterior position. × 1.

PLATE 6

Figs. *A, E, F*. Conidia of *Massospora cicadina*. × 1048.

Figs. *B, C, D*. Conidia germinating. × 568.

Fig. *G*. Optical cross section of conidium showing its thick wall. × 1048.

Fig. *H*. A group of conidiophores. × 568.

Figs. *I, J, K, L*. Selected conidiophores showing the method of formation of the conidia. × 568.

Fig. *M*. Gourd shaped hyphal bodies associated with the resting spore condition. × 268.

Figs. *N, O*. Young resting spores with hyphal bodies attached. × 268.

Figs. *P, S*. Young resting spores with hyphal bodies attached. × 532.

Fig. *Q*. A stage in resting spore development intermediate between those shown in Figs. *P* and *T*. × 532.

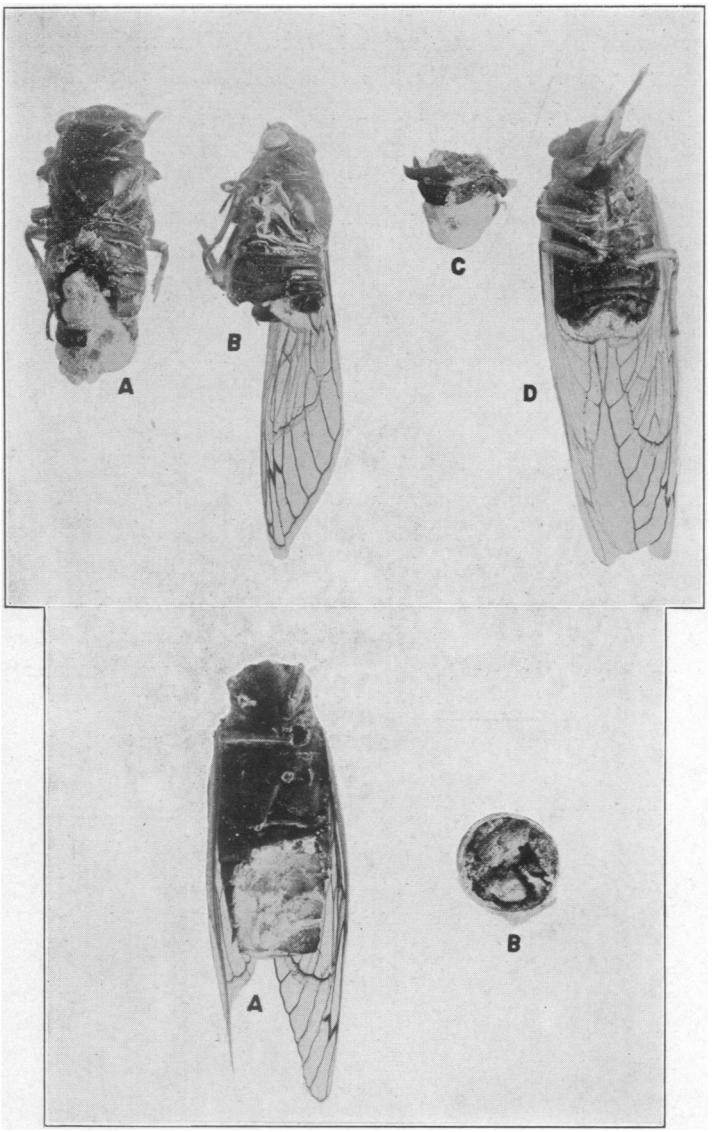
Fig. *R*. Apparently an encysted hyphal body. × 532.

Fig. *T*. Mature resting spore. × 568.

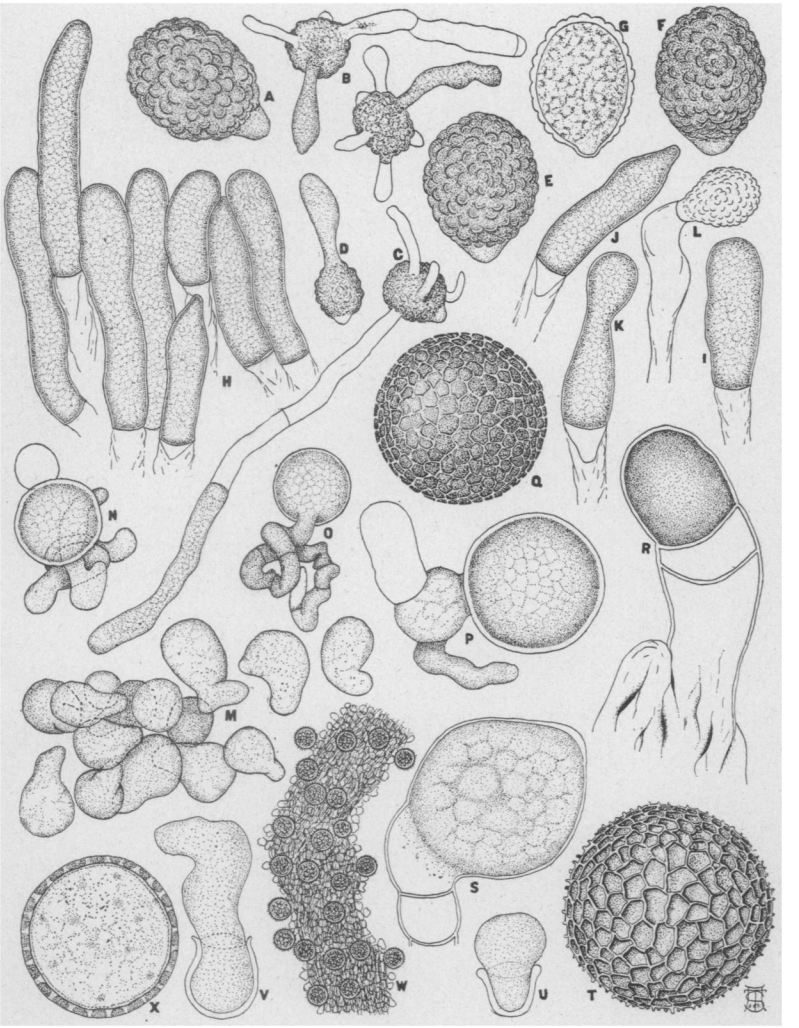
Figs, *U, V*. Hyphal elements of unknown origin and function found associated with the resting spore condition. × 568.

Fig. *W*. A portion of one of the tube-like genital organs, showing resting spores and hyphal bodies adhering. × 62.

Fig. *X*. Optical cross section of a resting spore in about the stage of development shown in Fig. Q. × 532.



1. (Above.) CONIDIA ON TIBICINA
2. RESTING SPORES ON TIBICINA



MASSOSPORA CICADINA Peck